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There is at the present time one international publication devoted entirely to cecidology, *Marcellia*, now in its eighth volume, edited by Dr. A. TROTTER, Avellino, Italy, which publishes original papers and also current bibliography.— MEL. T. COOK, *Delaware Agric. Exper. Station, Newark*.

FIXING AND STAINING TANNIN IN PLANT TISSUES WITH NITROUS ETHERS

(WITH EIGHT FIGURES)

Immature dates exposed to the vapor of amyl or ethyl nitrite to stimulate premature ripening were observed to turn dark brown very rapidly. This was found to result from the staining of the giant tannin cells which occur near the cuticle. After several hours' exposure, hard tannin grains were formed in the tannin cells of green dates and persimmons, which had

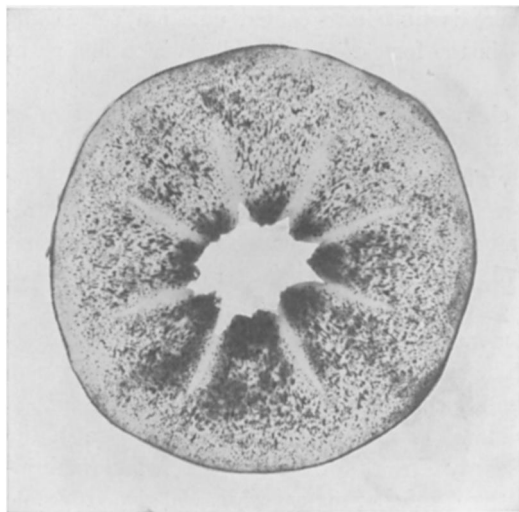


FIG. 1.—Japanese persimmon.

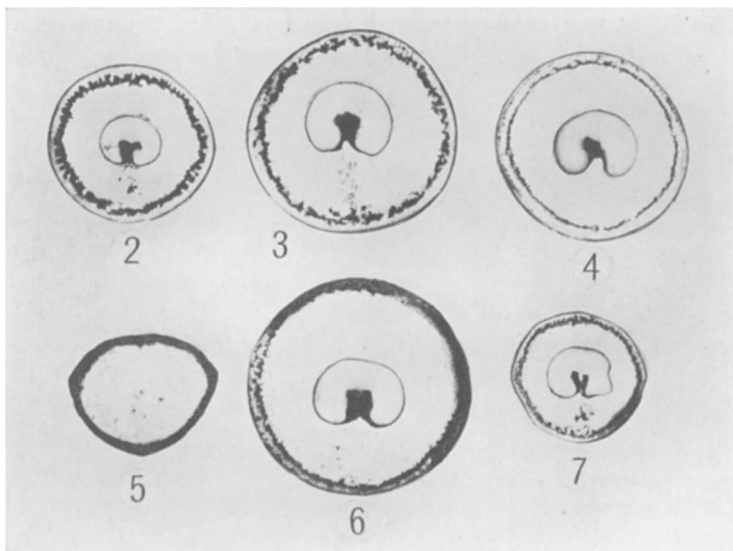
the same physical characters as the grains occurring naturally in ripe fruit; they could be separated quite pure by gravity in water, and when pressed beneath the cover glass fractured like grains of gelatin.

The juice of the unripe date gives a dense brown precipitate with ethyl or amyl nitrite or with sodium nitrite and free acid. Persimmon juice gives an intense deep wine-red color, but no precipitate.

Tannic and gallic acids

give yellow, while phloroglucin and some other higher phenols give a red color very similar to that given by date or persimmon juice. Phloroglucin also gives a precipitate, but it does not correspond in appearance with that given by date juice. The other higher phenols also give yellow or red color reactions with nitrous ether. The gummy matter, pectin, etc., precipitated from the juice by alcohol carries with it much of the substance that reacts with the nitrite, and the color given by this pre-

cipitate increases by repeated precipitations. Date juice obtained under low pressure continues for many weeks to give the ethyl nitrite reaction, as also the reactions with formaldehyde and ferric chlorid, but juices obtained under very high pressure, after standing, lose the property of giving these reactions, excepting that with ethyl nitrite a very much lighter colored precipitate is formed. DAVY⁵ found the color reaction between gallic acid and nitrous acid very delicate, and applied it to the colorimetric estimation of nitrites.



FIGS. 2-7.—*Fig. 2*, Deglet Noor date; *fig. 3*, Oga de Bedreschen; *fig. 4*, Birket el Haggi; *fig. 5*, seedling unfertilized; *fig. 6*, same seedling fertilized; *fig. 7*, M'Kenti-chie Degla.

It is thus not easily proven whether the precipitate formed in the date juice is due to date tannin alone or whether other substances enter the reaction. Tannin or its derivatives, however, are essential to the reaction, as shown by the following evidence. Only those cells in the date are stained by ethyl nitrite which have been shown to carry tannin by the ferric chlorid, ammonium molybdate, and cupric acetate reactions. Ripe date juice no longer gives the reaction, and immature fruits lose their astringency after very moderate treatment with ethyl nitrite. Juices treated with lead subacetate, gelatin acid salt solution, and hide powder no longer

⁵ Through J. DEKKER, *De Looistoffen II*. p. 44. Amsterdam. 1908.

give the reaction. The lead subacetate precipitate, as also the gelatin and hide powder residues, is stained very dark brown by the reagent.

The method proves very convenient in studying the distribution of tannin in fruits and other parts of plants. Tissues which can be distinguished only with difficulty in the unstained hand section become very sharply differentiated after treatment with nitrous ether vapor. For example, a piece of the woody fruit stem of the date stained in this way reveals a very narrow tannin zone near the cuticle, corresponding very closely to the more prominent one in the fruit. In thin section this zone resolves into rather widely separated, very small, dark-colored cells. A large Japanese persimmon weighing one-half pound can be completely

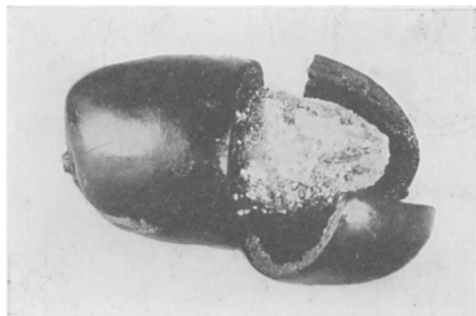


FIG. 8.—Deglet Noor date showing sharp separation of tannin layer from inner flesh after ethyl nitrite treatment.

stained in 24 to 48 hours without any abrasion of the cuticle. The process offers the further advantage that the tannin is deposited in the cells where it occurs, and thus eliminates all danger of carrying soluble tannin across the face of a section. In the date the outer tannin-bearing tissue detaches and may be separated very easily and sharply from the inner tannin-free tissue after pro-

longed exposure to ethyl nitrite. In some fruits, certain cells stain violet or lilac instead of brown, while others take no stain whatever until after prolonged treatment. This is notably true in the wild persimmon, which stains brown very readily just beneath the skin and again at the center of the fruit, with the exception of occasional cells interspersed through the flesh. Further exposure to the vapor of nitrous ether stains all the tannin cells uniformly brown. It is not evident whether this phenomenon depends entirely on permeability of the cell walls, or whether there is an actual difference in chemical composition of the cell contents.

For laboratory use a 20 per cent. alcoholic solution made by diluting the 90 per cent. commercial nitrous ether is recommended. Amyl nitrite may be used, but is disagreeable to work with. Ordinary sweet spirits of niter, which contain about 4 per cent. ethyl nitrite, may be used, but will require much longer exposure.—A. E. VINSON, *Arizona Agric. Exper. Station, Tucson, Arizona.*